Is FISH Painting an Appropriate Biological Marker for Dose Estimates of Suspected Accidental Radiation Overexposure? A Review of Cases Investigated in France from 1995 to 1996

Irène Sorokine-Durm, Valérie Durand, Annie Le Roy, Nathalie Paillole, Laurence Roy, and Philippe Voisin

Institut de Protection et de Sûreté Nucléaire, Département de Protection de la Santé de L'Homme et de Dosimétrie, IPSN, Fontenay-aux-Roses cedex, France

From 1995 to 1996 about 15 people suspected of being overexposed to ionizing radiation were referred to the Institute for Nuclear Safety and Protection in Fontenay-aux-Roses, France, for investigation by chromosome aberration analysis. Biological estimates of accidental overexposure were first obtained by scoring radio-induced unstable structural chromosome aberrations (dicentrics, centric rings, and fragments) in peripheral blood lymphocytes. For dose estimates, the yield of these chromosomal aberrations observed in 500 metaphases was compared with the laboratory dose–response relationship established from human blood irradiated *in vitro* (γ-rays, ^{60}Co, 0.5 Gy/min). To extend the possibilities of detecting DNA damage from earlier exposures by visualizing stable chromosome aberrations, chromosome painting by fluorescence *in situ* hybridization (FISH painting) was developed using a cocktail of three composite whole human chromosome-specific DNA probes (numbers 2, 4, and 12). A laboratory calibration curve for scoring terminal and/or reciprocal translocations was established for the same radiation quality and dose rate as those used for conventional cytogenetics (γ-rays, ^{60}Co, 0.5 Gy/min). For dosimetry purposes, it was also important to verify whether FISH painting could be applied to each human blood sample assessed for conventional expertise. For each individual, 2000 metaphases were scored for the presence or absence of reciprocal and terminal translocations. We present here a comparison between the results obtained by the two technologies for each of the cases studied separately. We describe their similarities or differences and discuss the suitability of using FISH painting for routine expertise analysis. — *Environ Health Perspect* 105(Suppl 6):1427–1432 (1997)

Key words: suspected radiation overexposure, chromosomal aberrations, dosimetry, conventional cytogenetics, FISH painting

Introduction

This paper considers the possibility of using the detection of stable chromosome aberrations by a fluorescence *in situ* hybridization technique (FISH painting) to estimate doses in cases of various accidental overexposures to ionizing radiation, which generally occur at low doses. Suspected overexposure to ionizing radiation is usually estimated by the number of unstable chromosome aberrations [dicentric (Dic) and centric rings] in peripheral lymphocytes of exposed individuals (*1*). The data obtained are then calibrated against a standard dose–response curve established after analysis of human lymphocytes exposed *in vitro* (*2*). However, Dic chromosomes are unstable with time after exposure (*3,4*) and a biological dosimetry based on their detection alone has limitations with regard to past overexposure. Problems may be encountered in dose reconstruction when the time between exposure and analysis is considerable or even unknown. On the other hand, it appears that translocations persist for many years after exposure and that their scoring may be an indication of past overexposure. FISH painting using whole human chromosome-specific DNA probes has opened new possibilities for detecting some interchromosomal exchanges (i.e., translocations, insertions) using a cocktail of composite DNA probes specific to some chromosomes (*5,6*). The data obtained by the analysis of only a few chromosomes (the painted ones) generally are scaled up to full genomic frequency by assuming a random distribution of break points. FISH painting, therefore, provides easy identification and classification of radiation-induced chromosome aberrations (*6–16*).

In cases of retrospective dose estimation translocation frequencies must also be calibrated against standard dose–response curves established *in vitro* using the same experimental protocols. This is particularly important because interlaboratory variabilities are suspected and a recent report clearly shows that care must be taken when interpreting FISH data from more than one laboratory (*17*).

Consequently, it appears that the best way to study the utility of FISH painting for dose assessment if overexposure is suspected is: *a*) to compare the scoring data obtained using this technique with those obtained by conventional cytogenetics for each case of accidental overexposure; and *b*) to establish an *in vitro* standard curve for translocation scoring using a quality of radiation and dose rate similar to those used for the laboratory reference curve for Dic scoring. This paper presents our preliminary results in this area of research.

Methods

In Vitro Irradiation Procedure, *in Vivo* Sampling, and Lymphocyte Culture

To establish the dose–effect reference curve, heparinized whole-blood samples from healthy donors were irradiated in a

This paper is based on a presentation at the International Conference on Radiation and Health held 3–7 November 1996 in Beer Sheva, Israel. Abstracts of these papers were previously published in *Public Health Reviews* 24(3–4):205–431 (1996). Manuscript received at *EHP* 7 April 1997; accepted 17 July 1997.

Address correspondence to Dr I. Sorokine-Durm, Institut de Protection et de Sûreté Nucléaire, Département de Protection de la santé de l'Homme et de Dosimétrie, Section Autonome de Radiobiologie Appliquée à la Médecine, IPSN, B.P. 6, F-92265, Fontenay-aux-Roses cedex, France. Telephone: 33 1 46 54 95 47. Fax: 33 46 54 84 67. E-mail: irene.sorokine@ipsn.fr

Abbreviations used: BrdU, 5-bromodeoxyuridine, thymidine analogue; DAPI, 4',6-diamidine-2'-phenylindole dihydrochloride; Dic, dicentric; EC, cells containing visible complex exchange; FISH, fluorescence *in situ* hybridization; FITC, fluorescein isothiocyanate; FPG, fluorescence plus Giemsa technique; Gy, gray; Ins, insertion; IPSN, Institute for Nuclear Safety and Protection; RX, X-rays; 2×SSC, saline–sodium citrate buffer concentrated 2-fold; TR, reciprocal translocation; TT, terminal translocation; (TR + TT) eq, genomic estimated translocations.

water bath at 37°C with 1.25 MeV γ-rays from a ^{60}Co source (ICO 4000, Institute for Nuclear Safety and Protection [IPSN], Fontenay-aux-Roses, France). Samples were irradiated up to 4 Gy at a dose rate of 0.5 Gy/min. Blood samples were then maintained at 37°C for 2 hr after irradiation to allow DNA repair. Control and irradiated whole-blood sample cultures were carried out in RPMI 1640 medium for 48 hr according to standard cytogenetic procedures (*1*). A demecolcine block (0.1 μg/ml, Life Technology, Paisley, Scotland, UK) was done during the two last hours. Chromosome slides were obtained after classical hypotonic shock (0.075 M KCl) and fixation of lymphocyte metaphases in methanol/acetic acid (3/1, v/v). For *in vivo* expertise, blood samples (2×5 ml) were collected during the medical examination of the patient by antecubital venipuncture in tubes containing lithium heparin as an anticoagulant (Becton Dickinson, Pont-de-Claix, France). Blood samples reached the laboratory within 24 hr of sampling. Whole-blood cultures were immediately carried out in duplicate, as explained above, and in the presence of 5-bromodeoxyuridine (BrdU) to score unstable chromosome aberrations in first metaphases only. Classical fluorescence plus Giemsa technique was used for conventional scoring (*1*). Slides kept for FISH were stored at –20°C with desiccant until use.

Fluorescence *in Situ* Hybridization

The hybridization protocol was applied according to Pinkel et al. (*5*) with some modifications. In brief, slides containing target DNA were dehydrated in ethanol series before a ribonuclease A treatment (1 mg/ml in 2-fold concentrated saline–sodium citrate buffer [2×SSC], 1 hr at 37°C, Boehringer Mannheim, Meylan, France) followed by a pepsin digestion (0.005% in HCl 0.01 N, 10 min at 37°C, Boehringer). The hybridization mixtures containing human whole chromosome-specific DNA probes (chromosomes 2, 4. and 12, Vysis, Voisins le Bretonneux, France) were premixed with unlabeled human competitive placental DNA enriched for repetitive DNA sequences (Cot 1 DNA) and incubated at 37°C for 1 hr 30 min. Target DNA was denaturated 3 min at 70°C in solution (70% formamide, 2×SSC). DNA probes were then deposited on the prewarmed (10 min, 56°C) denaturated slides and hybridization occurred overnight in a humid chamber at 37°C. Posthybridization washings were done according to standard Vysis protocol with shortened incubation times (3 min). Chromosomal DNA was counterstained with 4′,6-diamidine-2′-phenylindole dihydrochloride ([DAPI] Sigma, Saint Quentin Fallavier), diluted in an antifade solution, and visualized under a fluorescence microscope.

Estimated Genomic Frequency of Aberrations

Estimation of the genomic translocation frequency was carried out using the formula proposed by Lucas et al. (*6,7*), ($Fp = 2.05\ fp(1-fp)Fg$), which links the observed translocation frequency on the painted chromosomes (Fp) to the total genomic translocation frequency (Fg) according to the fraction of the genome painted (fp). For the chromosomes painted in this study (2, 4, and 12), 18.6% of the genome was hybridized corresponding to a detection efficiency of 31%. The total number of metaphases scored at each dose was corrected (see "Cell Equivalent" in Table 1) to correspond with the amount of information that would have been available if aberrations had been scored by G banding. This process led to a genomic estimation of translocations.

Criteria for Aberration Scoring and Curve Representations

Conventional scoring was performed on an Optiphot microscope (Nikon, Micromècanique, Evry, France). Only complete cells (i.e., 46 centromeres) in first division were analyzed for the presence of Dics (Nikon×100 objective, NA 1.25, oil). FISH slides were analyzed with a Microphot-FXA (Nikon) fluorescence microscope (mercury lamp, 100 W) equipped with Nikon PlanApo×60 objective (NA 1.4, oil) combined with filter blocks for simultaneous observation of fluorescein isothiocyanate (FITC)/rhodamine (Nikon) or DAPI/FITC/rhodamine (Nikon). A simple pass DAPI filter (Nikon) was used to verify chromosome shapes. For FISH painting, only complete-looking metaphases that had complete painted patterns were considered.

Cells were analyzed according to the following criteria. A bicolored chromosome exhibiting a single centromere in the painted (red or green) part was classified as reciprocal translocation(s) (TR). This translocation was complete if its reciprocal bicolored counterpart was observed or it was incomplete (TRi) if only one bicolored monocentric chromosome was seen accompanied by the corresponding painted fragment. A bicolored chromosome with the centromere located in the nonpainted part of the chromosome (i.e., DAPI colored) was termed terminal translocation (TT). Generally, a TT was always accompanied by a painted truncated chromosome. Insertions (Ins) looked similar to a painted chromosome piece inserted in a nonpainted one. Two-color chromosomes with two or more

Table 1. Comparison between the yields of unstable and stable chromosome aberrations produced in blood peripheral lymphocytes by *in vitro* irradiation with γ-rays from ^{60}Co at a dose rate of 0.5 Gy/min. Chromosome aberrations were scored by conventional cytogenetics and FISH painting.

Conventional cytogenics				FISH painting (chromosomes 2, 4, and 12)									
Dose, Gy	Cells scored	Dic	Yield of Dic	Dose, Gy	Cells scored	TR	Ins	TT	TR+TT	EC	Cell equivalent	TR eq, no per cell	(TR + TT) eq per cell
0.00	2305	1	0.0004	0.00	2061	0	0	0	0	0	640	0	0
0.10	2005	18	0.0009	–	–	–	–	–	–	–	–	–	–
0.24	2028	22	0.0108	–	–	–	–	–	–	–	–	–	–
0.33	2010	23	0.0114	0.50	660	2	0	3	5	0	205	0.0098	0.0244
0.69	1501	61	0.0406	–	–	–	–	–	–	–	–	–	–
1.00	869	89	0.1024	1.00	1126	22	0	8	30	1	349	0.0630	0.0859
1.35	1005	160	0.1592	–	–	–	–	–	–	–	–	–	–
1.54	505	96	0.1901	–	–	–	–	–	–	–	–	–	–
2.00	1366	430	0.3148	2.00	322	13	2	9	22	2	100	0.1302	0.2204
3.00	794	476	0.5995	3.00	263	35	2	14	49	4	82	0.4268	0.5976
4.00	646	609	0.9427	4.00	316	56	4	40	96	1	98	0.5717	0.9800
Total	13,024	–	–		4748	128	8	74	202	8	1472	–	–

centromeres were considered multicentrics. Cells containing visible complex rearrangements (EC), i.e., those arising from the interaction of a minimum of three breaks on a minimum of two chromosomes (*18*), were analyzed and scored separately. For *in vivo* dose estimation, Dics were scored in 500 metaphases per individual and translocations were scored in 2000 metaphases per case.

To establish a dose–response reference curve, a linear–quadratic regression model was applied to each point and best fitted curves obtained using an iteratively reweighted regression calculation (Sigma Plot, Jandel Scientific, San Raphael, CA) assuming that chromosome aberrations follow a Poisson law distribution.

Results

In Vitro Studies

Table 1 shows data obtained by scoring unstable and stable chromosome aberrations induced *in vitro* in human lymphocytes by γ-rays from a ^{60}Co source at a dose rate of 0.5 Gy/min, as explained in "Methods." Results of Dic yields scored after conventional staining are listed in Table 1, which also shows the scoring by FISH painting of TR (complete and incomplete), TT, and Ins involving the painted chromosomes 2, 4, and 12. In this preliminary work, simple-looking complete and incomplete exchanges (i.e., bicolor chromosomes with only one color junction) or insertions (two color junctions) were considered. Exchanges between two painted chromosomes were scored as single events. Cells containing visible EC were scored separately and not included in the total data. To compare the yields of stable chromosome aberrations with those of the unstable aberrations, all observed frequencies were genomic estimated using the formula of Lucas et al. (*6,7*). So a full genome equivalent cell number (cell equivalent) was calculated and the corresponding genomic estimated translocation number was termed (TR + TT)eq.

Data from Table 1 were used to establish three dose–response fitted curves (Figure 1). In this figure the dose–effect relationship obtained from Dic yields scored by conventional cytogenetics is compared to the one obtained from the TReq yields for blood samples irradiated *in vitro* in the same conditions. The third dose–effect curve corresponds to the genomic estimated yield calculated for all types of translocations (TR + TT)eq. In this study, the level of genome equivalent TR scored by FISH painting is lower than the level of Dics scored by conventional cytogenetics. But when all types of translocations are taken in account, the two curves, i.e., Dics and translocations, are similar.

In Vivo Studies

The cases of suspected overexposure to ionizing radiation referred to our laboratory from 1995 to 1996 can be grouped roughly into two main categories—professional (i.e., working with radioactivity or radioactive sources or close to them) and public (usually not using ionizing radiation). Among the group of professional workers almost 40% carried no personal physical dosimeter at the moment of the accident (data not shown). Individuals' blood samples were coded by a letter according to the date of their arrival at the laboratory (Table 2). One case was analyzed two times with blood sampling separated by 9 weeks (cases L1 and L2). The quality of radiation exposures involved in this study were from diverse origins and modes, as seen in Table 2. The cases of exposure to X-ray(s) (RX), ^{192}Ir, or ^{137}Cs were external—acute and localized for cases A, E, F, O, or recurrent but heterogeneous for cases B, C, I. Two individuals (L and N) were involved in internal contamination by ^{90}Sr or ^{35}S. Two other individuals (G and H) were suspected of external heterogeneous exposition to ^{226}Ra, with a possible internal contamination for one (G). The contaminated area for J and K corresponds to undefined possibilities of exposure for two people who stayed more than 30 months in geographical zones that possibly were contaminated. All the cases were different and it was impossible to arrange them by groups according to the type of irradiation.

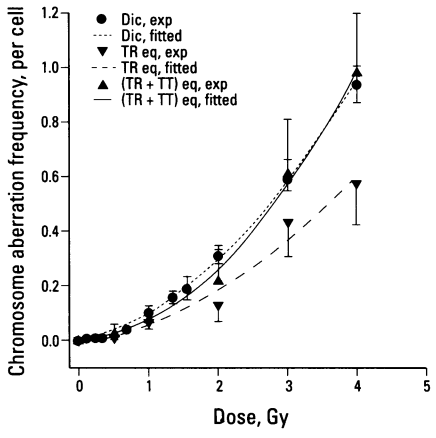

Figure 1. Dose–effect relationships obtained by conventional cytogenetics and by FISH painting (^{60}Co, dose rate of 0.5 Gy/min). Dics were scored using conventional cytogenetics; stable chromosome aberrations (TR and TT) were stained and scored using the FISH-painting technique (chromosomes 2, 4, and 12). Vertical bars represent the 95% Poisson CI. Lines are fitted curves using an iteratively reweighted least square regression as a Poisson chromosome aberration distribution.

Consequently, it was proposed to classify these cases in four groups, I to IV, according to the delay between the suspected overexposure and the time of the analysis. This classification was chosen to test the efficiency of translocation detection by FISH painting, which was used as a bioindicator of past exposure. The results obtained by FISH were compared with those obtained using conventional cytogenetics (Dics scoring) for the same patients. For this purpose, a minimum of 2000 cells per individual were scored by FISH painting to detect stable chromosome aberrations (except case A, where only 861 cells could be found). This number corresponds to a full genome equivalent cell number of about 620 cells and the statistical uncertainty level became reliable with the 500 cells visually scored for Dics by conventional cytogenetics. In general, even for conventional cytogenetics or for FISH painting, cells carrying only one aberration were the most commonly found, especially for *in vitro* γ-ray irradiation at low doses.

However, sometimes cells with multi-aberrations were found in accidental overexposure cases, even at low doses estimated by physic dose reconstruction when possible. This was often seen in cases of radionuclides with beta-emission components. For this reason, some cases in Table 2 are scored two ways to distinguish two types of analysis: scoring excluding (single letter) or including (double letter) cells having many aberrations. This was true for case L, which was scored either taking into account one cell carrying many Dics (case LL1) or not (L1). The same procedure was used for cases that had complex exchanges detected by FISH painting (EC cells in Table 2). When possible, complex exchanges were scored by transferring the complex patterns into a simple-looking base-type translocation (i.e., TR or TT). This was true for cases LL2-L2, E-EE, and A-AA. Case C had one cell carrying an exchange that was too complex; the cell was excluded from the results. To facilitate comparison between the Dic and the genomic estimated translocation yields, Table 2 gives the ratio between these two types of aberrations per cell. These ratios represent the disappearance of unstable

Table 2. Analyses of suspected accidental overexposure cases according to irradiation history and the corresponding yield of unstable and stable chromosome aberrations scored by conventional cytogenetics and FISH painting.

Group	Delay between exposure and analysis	Case Coding	Radiation type and exposure mode	Age at blood sampling, years	Dic per cell, $\times 10^{-3}$	Multi Dic,[a] no	Complex exchange, no	TR eq per cell, $\times 10^{-3}$	(TR+TT) eq per cell, $\times 10^{-3}$	Dic/ TReq, ratio	Dic/ (TR+TT) eq, ratio
I (short delay)	1 to 8 days	F	^{192}Ir (ext)	46	5.8	0	0	8.1	9.7	0.72	0.60
		LL1	^{90}Sr (int)	8	12	1	0	1.4	1.4	8.57	8.57
		L1			4	0	0	1.4	1.4	2.86	2.86
		O	RX (ext)	60	2	0	0	4.8	8.1	0.42	0.25
II (medium delay)	2 to 2.5 months	AA	^{137}Cs (ext)	46	8	0	1	3.74	30.0	2.14	0.27
		A			8	0	0	3.74	22.5	2.14	0.36
		DD	^{192}Ir (ext)	29	11.8	1	0	2	6.1	5.90	1.93
		D			5.9	0	0	2	6.1	2.95	0.97
		EE	RX (ext)	40–45	5.8	0	1	7	10	0.83	0.58
		E			5.8	0	0	5	6	1.16	0.97
		LL2	^{90}Sr (int)	8	2	0	1	8.1	9.7	0.25	0.21
		L2			2	0	0	3.3	4.8	0.61	0.42
		M	Cont area (ext)	32	0	0	0	6.4	6.4	–	–
		N	^{35}S (int)	26	2	0	0	2.5	3.7	0.80	0.54
III (long delay)	0 to 10 years	B	RX (ext)	37	0	0	0	6.7	9.4	–	–
	3 to 10 years	C	^{192}Ir (ext)	58	6	0	0	4.8	12.9	1.25	0.47
	0 to 30 years	G	^{226}Ra (ext?)	45–50	0	0	0	1.6	6.5	–	–
	0 to 4 years	H	^{226}Ra (int?)	40–45	0	0	0	1.6	1.6	–	–
	0 to 10 years	I	RX (ext)	40–50	0	0	0	0	0	0	0
IV (protracted)	5 months	J	Cont area (int?)	35	0	0	0	8.1	11.3	–	–
	5 months	K	Cont area (int?)	30	0	0	0	3.2	3.2	–	–

Abbreviations: ?, supposed to be; Cont area, contaminated area (from some regions in the East countries); ext, external exposure; int, internal exposure by contamination.
[a]Cell with more than one dicentric (Multi Dic). Multi Dic is the number of cells having more than one unstable chromosome aberration. Each individual is coded by a letter. A single letter represents scoring data that do not take into account those cells having more than one aberration. Double letters represent scoring taking these kind of cells into account. One case, L, was analyzed for two periods; a number near the letter distinguishes the two scorings.

chromosome aberrations with time after exposure, but the data obtained are often dissimilar. In groups I and II, where the delay between suspicion of overexposure and analysis is theoretically short enough so there is no Dic loss, the genomic estimated total translocation yield (TT+TR)eq often exceeds the Dic frequency. However, case L1 surprisingly presents more Dics than translocations, even when the cell carrying many Dics was misscored (L1). When a new analysis was performed with a new blood sample 9 weeks later (L2-LL2), however, the observed ratio was the opposite, i.e., there were more translocations than Dics. Conversely, except for case C, no Dics were found for groups III (long delay) and IV (protracted), although some stable aberrations persisted at a level higher than background level (see Table 1).

Figure 2 presents three interesting examples of aberrations stained by FISH painting and visualized by fluorescence microscopy. Interpretations of all exchanges are given to the left of each photograph. Figure 2A shows a complete TR of chromosome 12 with a nonpainted one found in an overexposed patient. Figure 2B is an example of a complete TR between two painted chromosomes of another overexposed individual (numbers 4 and 12). Figure 2C illustrates an incomplete TRi between chromosomes 2 and 4.

Discussion

The primary purpose for using biological dosimetry in cases of suspected radiation overexposure is to help the medical staff devise a therapeutic strategy. Therefore, it is necessary to ascertain as quickly as possible the answers to such crucial questions as, "Was this person really overexposed to ionizing radiation?" and, "What was the dose estimation?" This paper describes the procedures we used in an attempt to answer these questions. Actually, the yield of unstable chromosome aberrations in phytohemagglutinin-stimulated human peripheral blood lymphocytes provide the most reliable biological indicator, provided the delay between irradiation and analysis does not exceed a few weeks to a few months (*1*). A gradual decrease in the frequency of cells carrying Dics generally is reported with increasing time after exposure (*4*). Some contrary examples were reported; for example, the presence of cells carrying unstable aberrations detected decades postirradiation in atomic survivors (*3*) or the case of some Thorotrast patient analysis [α-particle emission (*19*)], but these cases are rare. Thus, the presence of several Dics certainly is useful information in analyzing exposure status. The correspondence between Dic yield and dose estimates could be obtained through a calibration curve, which must be established *in vitro* under the same experimental conditions as those for analysis. This was the first step of our analysis and Figure 1 shows the laboratory reference curve obtained for γ-rays of ^{60}Co at 0.5 Gy/min. In more than half the cases we analyzed, the delay between exposure and the cytogenetic analysis was more than two months so Dic scoring by conventional cytogenetics might present some limitations. The introduction of FISH techniques in our laboratory has allowed us to extend the spectrum of chromosome-type aberrations to stable ones that can be analyzed. It is reasonable to suggest that this

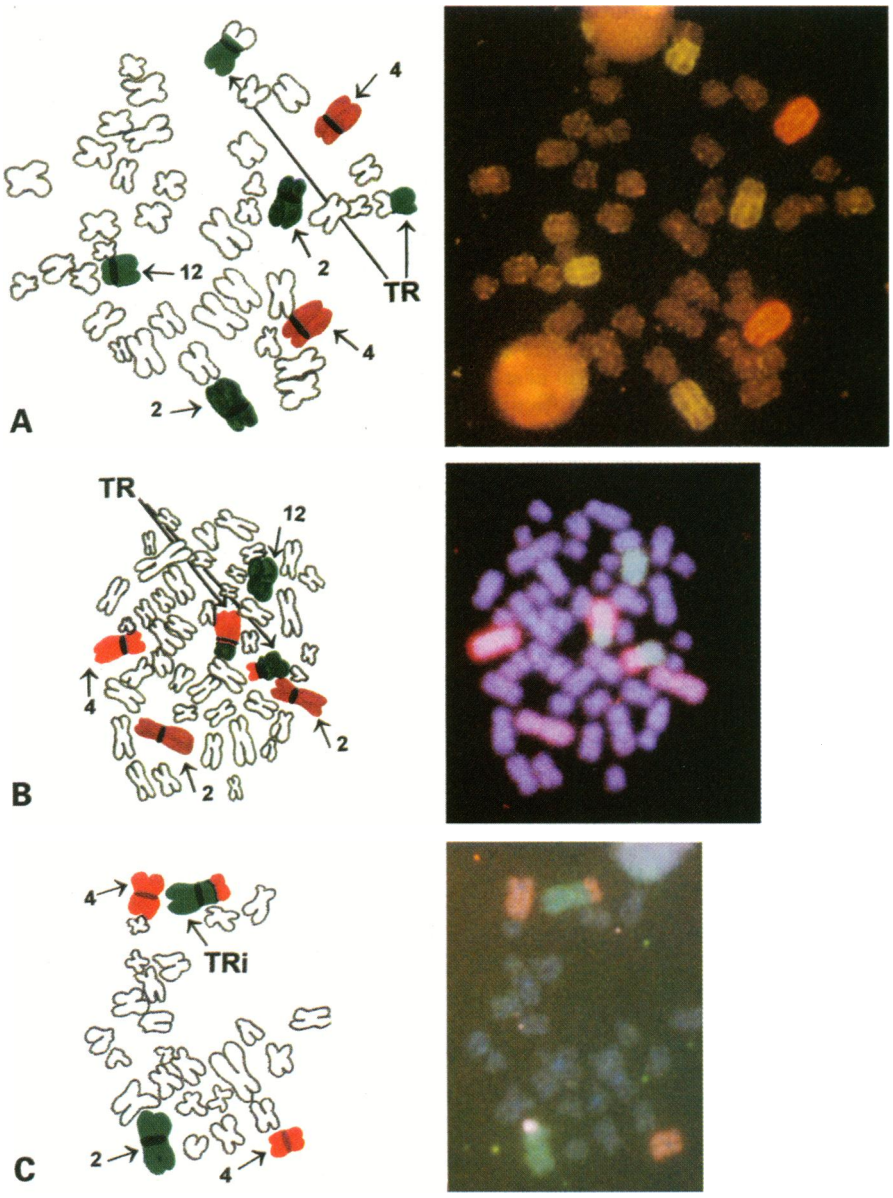

Figure 2. Three examples of stable chromosome aberrations found after FISH painting using two- or three-color composite whole-human DNA probe cocktail (2, 4, and 12). Two cases (photos *A*, *B*) were accidental overexposures and one case (photo *C*) is *in vitro* irradiation with γ-rays from ^{60}Co. Photo *A*: Chromosomes 2 and 12 are labeled with SpectrumGreen (Vysis) and chromosomes 4 are labeled with SpectrumOrange (Vysis). Photo *B*: Chromosomes 2 and 4 are labeled with SpectrumOrange (Vysis) and chromosomes 12 are labeled with SpectrumGreen (Vysis). Photo *C*: Chromosomes 2 are labeled with SpectrumGreen (Vysis) and chromosomes 4 are labeled with SpectrumOrange (Vysis). Diagrams to the left of the photos represent each metaphase and show details of painted chromosomes and corresponding aberrations. Photos were taken with Kodak Ektachrome color 1600 Aza film (Panther P1600, Kodak limited, England) at ×1000 magnification without digital processing.

new technology could improve dose reconstruction in cases of past overexposure and probably be useful in new and old dose estimates as well.

What are the limitations in cases of low-dose exposure? To find answers to these questions we established a calibration curve using three-color painting for three chromosomes (2, 4, and 12) that differed enough in length to be easily distinguishable. This choice of chromosomes represents a mean of chromosome radiosensitivity: one seems to be more often involved in the formation of symmetrical exchanges (number 4), another seems to be less frequently involved (number 2), and the third (number 12) seems to have a medium involvement, as shown recently by Knehr et al. (*16*). Figure 1 presents data for this curve, which needs more scoring in the low-dose range to be really usable for dose estimation assay. Nevertheless, preliminary results show that the number of genomic estimated total translocations [i.e., (TR + TT)eq] is similar to the number of dicentric ones but also that, in our case, the number of TR is lower. Even though the theoretical prediction on the equality of radiation-produced dicentrics and translocations (*20*) is not supported by many publications, further analyses are being done in our laboratory to resolve the apparent discrepancy between the number of TR scored by FISH painting and the number of dicentrics scored by conventional cytogenetics.

To eliminate any statistical bias, the applicability of FISH painting on *in vivo* accidental exposure was then tested using the same cocktail of DNA probes as the one used in establishing a reference curve. Our experience in the field of accidental overexposure shows that overexposure cases are rarely similar (Table 2). Differing radiation qualities (γ-rays, X-rays, β emission), modes of exposure (acute, heterogeneous), and possibly dose rates were involved. Consequently, the findings given in this study must be considered preliminary because the number of expertise cases is too small to draw clear conclusions. Nevertheless, classification of the scoring data in the four groups according to the supposed delay between suspicion of irradiation and the analysis (Table 2) leads to some interesting observations, which are discussed below. First, even though the delay was short between suspicion of overexposure and analysis (Table 2, Group I), no conclusion was possible because the number of cases was too small. It must be noted that case L apparently presented more Dics than translocations just after irradiation (LL1-L1). Two months later, however, the number of Dics decreased and the number of translocations appeared to have increased (LL2-L2). This internal exposure resulted from an accidental injection of strontium and the decrease of Dics might be because of the delay between elimination of the strontium (more than a week) and the moment of the second analysis (9 weeks later). Conversely, the apparent increase of stable chromosome aberrations as translocations might have occurred while the strontium was still in the body (more than 1 week) and may not have decreased in the 2 months before the

second analysis. The second group (Table 2) seems more homogeneous and, as expected with a longer delay period, the translocation level generally is higher than the Dic level. However, there are two exceptions. First is case A, which exhibits many fewer TR than TT, giving a higher value for Dics when compared with those for the TR. We cannot explain this observation. The second case (D) had localized irradiation to both hands. If the cell carrying a lot of Dics is taken into account (DD), the number of Dics is higher than the number of translocations. Nevertheless, our dose estimate supports the known discrepancy between a very heterogeneous irradiation to the hands and the whole-body integrated dose given by blood lymphocytes. In the third group Dics seem to have disappeared if, indeed, they ever existed, except for case C. The history of this patient was unclear and no conclusion could be drawn. Moreover, the presence of translocations in this third group cannot be explained by a simple effect of age because case B is still young but has a high translocation level. In fact, the effect of age on translocation frequency remains a confounding variable, as explained in the report of Chung et al. (*21*). Group IV gathers two protracted cases of overexposure in a contaminated area over 3 years. Analyses were done 5 months after return to the noncontaminated area and show a translocation level apparently higher than the background one. Whereas no Dics were observed, a significant yield of stable aberrations was found. It is difficult to ascertain whether this level is attributable to the 3 years period in the contaminated area because we do not know the background translocation frequencies of these people before the suspicion of overexposure. Note that with our present reference curve no translocation was found in control samples. All these first observations point out the necessity of stable chromosome aberration analysis when the delay between exposure and analysis increases.

A second observation is that a better understanding of the population background with regard to such factors as life habits, working conditions, and environmental situations is essential before using FISH painting as a biodosimeter. It is also necessary to solve the problem of age before validating translocation scoring as a biological indicator of suspicion of *in vivo* exposure. Table 2 shows examples of 40- to 50-year-old people with only a few translocations.

This study constitutes a preliminary step in our process of defining the possibilities of FISH painting for biological dosimetry expertise. Because of the limitations of a number of cases, no clear conclusion could be reached. In actuality these data provide more questions than answers in the case of varied suspected accidental overexposure.

REFERENCES

1. IAEA. Biological Dosimetry: Chromosomal Aberration Analysis for Dose Assessment. Tech Rpt Ser No 260. Vienna:International Atomic Energy Agency, 1986.
2. Bender MA, Awa AA, Brooks AL, Evans HJ, Groer PG, Littlefield G, Pereira C, Preston RJ, Wachholz BW. Current status of cytogenetics procedures to detect and quantify previous exposure to radiation. Mutat Res 196:103–159 (1988).
3. Awa A, Sofuni T, Honda T, Itoh N, Neriishi S, Otake M. Relationship between the radiation dose and chromosome aberrations in atomic bomb survivors of Hiroshima and Nagasaki. J Radiat Res 19:126–140 (1978).
4. Bauchinger M, Schmid E, Braselmann H, Willich N, Clemm C. Time-effect relationship of chromosome aberrations in peripheral lymphocytes after radiation therapy for seminoma. Mutat Res 211:265–272 (1989).
5. Pinkel D, Straume T, Gray JW. Cytogenetics analysis using quantitative high-sensitivity fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938 (1986).
6. Lucas JN, Tenjin T, Straume T, Pinkel D, Moore D II, Litt M, Gray JW. Rapid human chromosome aberration analysis using fluorescence *in situ* hybridization. Int J Radiat Biol 56:35–44 (1989).
7. Lucas JN, Awa A, Straume T, Poggensee M, Kodama Y, Nakano M, Ohtaki K, Weier H-U, Pinkel D, Gray J et al. Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int J Radiat Biol 62:53–63 (1992).
8. Cremer C, Cremer T. Analysis of chromosomes in molecular tumor and radiation cytogenetics: approaches, applications and perspectives. Eur J Histochem 36:15–25 (1991).
9. Lichter P, Boyle AC, Cremer T, Ward DC. Analysis of genes and chromosomes by non-isotopic *in situ* hybridization. Genet Anal Tech Appl 8:24–35 (1991).
10. Natarajan AT, Vyas RC, Darroudi F, Vermeulen S. Frequencies of X-ray-induced chromosome translocations in human peripheral lymphocytes as detected by *in situ* hybridization using chromosome-specific DNA libraries. Int J Radiat Biol 61:199–203 (1992).
11. Gray JW, Pinkel D, Brown JM. Fluorescence *in situ* hybridization in cancer and radiation biology. Radiat Res 137:275–289 (1994).
12. Finnon P, Lloyd DC, Edwards AA. Fluorescence *in situ* hybridization detection of chromosomal aberrations in human lymphocytes: applicability to biological dosimetry. Int J Radiat Biol 68:429–435 (1995).
13. Tucker JD, Morgan WF, Awa AA, Bauchinger M, Blakey D, Cornforth M, Littlefield LG, Natarajan AT, Shasserre C. A proposed system for scoring structural aberrations detected by chromosome painting. Cytogenet Cell Genet 6:211–221 (1995).
14. Tucker JD, Lee DA, Moore II DH. Validation of chromosome painting. II: A detailed analysis of aberrations following high doses of ionizing radiation *in vitro*. Int J Radiat Biol 67:19–28 (1995).
15. Chung HW, Ryu EK, Kim YJ, Ha SW. Chromosome aberrations in workers of nuclear-power plants. Mutat Res 350:307–314 (1996).
16. Knehr S, Zitzelsberger H, Braselmann H, Nahrstedt U, Bauchinger M. Chromosome analysis by fluorescence *in situ* hybridization: further indications for a non-DNA-proportional involvement of single chromosome in radiation-induced structural aberrations. Int J Radiat Biol 70:385–392 (1996).
17. Moore DH II, Epstein L, Reeder J, Wheeless L, Waldman FM, NCI Bladder Tumor Marker Network. Interlaboratory variability in fluorescence *in situ* hybridization. Cytometry 25:125–132 (1996).
18. Savage JRK, Simpson PJ. FISH "painting" patterns resulting from complex exchanges. Mutat Res 312:51–60 (1994).
19. Tanaka K, Poop S, Fisher C, Van Kaicks G, Kamada N, Cremer T, Cremer C. Chromosome aberration analysis in atomic bomb survivors and Thorotrast patient using two- and three-colour chromosome painting of chromosomal subsets. Int J Radiat Biol 70:95–108 (1996).
20. Lucas JN, Chen AM, Sachs RK. Theoretical predictions on the equality of radiation-produced dicentrics and translocations detected by chromosome painting. Int J Radiat Biol 69:145–153 (1996).
21. Chung HW, Ryu EK, Kim YJ, Ha SW. Chromosome aberrations in workers of nuclear-power plants. Mutat Res 350:307–314 (1996).